A Pre-PhD Synopsis

"Impact of Bacterial Pathogens on Physiological and Nutritional Profile of *Cyprinus carpio* from Limboti Dam, Dist.Nanded (MS)"

स्वामी रामानंद तीर्थ मराठवाडा विद्यापीठ, नांदेड.

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Faculty of Science and Technology

By

Tamke Sneha Sanjay

Under Guidance of

Dr. R. D. Barde

Professor & Head,

Department of Zoology,

Shri Guru Buddhiswami Mahavidhyalaya, Purna (Jn.)

Tq. Purna, Dist. Parbhani-431511

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Abstract:

Due to its versatility, adaptability and economic value, *Cyprinus carpio* is a highly important species in inland aquaculture and plays a role in nutrient cycling and water quality management in polyculture system . However, under fluctuating environmental conditions and poor water quality—common in open reservoirs like Limboti Dam, Dist.Nanded—this species becomes susceptible to a variety of bacterial diseases. By means of extracellular virulence enzymes such as proteases, lipases, and lecithinases that compromise tissue integrity and disturb systemic physiological processes, pathogenic bacteria—especially *Aeromonas hydrophila*, *Edwardsiella tarda*, and *Pseudomonas fluorescens*—are known to cause considerable morbidity and death in cyprinid fishes.

This synopsis presents a multidisciplinary investigation aimed at isolating and characterizing bacterial pathogens infecting wild *Cypr*inus carpio populations in Limboti Dam, to evaluate their virulence through enzymatic profiling, and assess the associated physiological and nutritional consequences in the host. Following morphological, biochemical, and molecular identification of pathogens, the study will include quantitative assessment of haematological parameters (Hb, RBC, WBC, PCV), histopathological changes in key organs (gills, liver, kidney), and dietary changes (protein, lipid, carbohydrate, vitamin content) in infected versus healthy specimens. water quality parameters such as temperature, pH, dissolved oxygen, and ammonia will also be monitored to understand their influence on pathogen spread and and disease severity, highlighting their impact on host health.

The work intends to establish a scientifically confirmed correlation between bacterial infections and host health decline in *Cypr*inus carpio populations by including microbiological, physiological, nutritional, and ecological data. This study aims to address the current knowledge gap in reservoir fish health management in Maharashtra and offer baseline data for future disease surveillance, intervention plans, and environmentally friendly aquaculture methods in semi-natural water bodies.

Keywords: Bacterial Pathogens, Physical Change, Nutritional Status, Limboti Dam Reservoir, *Cyprinus carpio* .

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1. Introduction

India ranks among the top global producers of inland freshwater fish, with its aquaculture sector playing a vital role in national food security, rural livelihoods, and agricultural sustainability (Jacob et al., 2020). Originally from Danub River basin, *Cypri*nus carpio was brought into Indian aquaculture in the 1939 because of its tolerance to a wide range of environmental conditions , omnivorous feeding habits it fits for polyculture systems including Indian main carps such *Catla catla* and *Labeo rohita*. Particularly in underdeveloped-rural areas, low input requirements, tolerance for various conditions and ability to thrive on both natural and supplemental feeds makes it a reasonably affordable portion of sustainable aquaculture systems.

*Cypri*nus carpio is becoming more vulnerable to bacterial infections in open water systems despite its commercial significance and ecological compatibility; infections often remain undetected until mortality occurs. In freshwater habitats, bacterial infections are acknowledged as the most often occurring and economically destructive causes of fish disease. Mass mortalities, stunted development, skin ulcers, and systemic infections spanning many cyprinid populations have been linked to infections by species including *Aeromonas hydrophila*, *Edwardsiella tarda*, *Pseudomonas fluorescens*, and *Flavobacterium columnare* (Aziz and Abdullah, 2021). These pathogens either naturally occurring in the aquatic environment or opportunistically invade fish in conditions of immunosuppression, poor water quality, or environmental fluctuation.

Bacterial pathogens release a variety of extracellular enzymes, including proteases, lipases, and lecithinases that break down host tissues and enable more thorough penetration. These virulence factors are known to disrupt host defenses, harm epithelial linings, and change internal physiological balance are these virulence factors. Often reflecting immunological dysfunction and poor oxygen delivery, systemic infections are shown in changed hematological profiles including reduced hemoglobin, suppressed red blood cell count, and leukocytosis or leukopenia. Furthermore, shown to influence metabolic activities in fish are chronic bacterial infections, which directly impact the health, market quality, and post-harvest nutritional value of the fish by causing catabolism of muscle tissue, lipid depletion, and reduced levels of essential nutrients including carbohydrates and vitamins (Ali et al., 2022).

Found in Nanded district, Limboti Dam is a freshwater reservoir used for fishery and irrigation. It provides fish for both domestic and commercial use, therefore supporting a significant share of

local fisheries-based revenue. But Limboti Dam lacks regular fish health monitoring and disease surveillance, much like many semi-managed inland water bodies in India. Though there is anecdotal evidence of illness outbreaks, no systematic microbiological analysis or nutritional profiling study has been done on commercially significant species like *Cypri*nus carpio in this reservoir. Further raising the possibility of microbial growth and stress-induced disease in fish populations are the great fluctuations in water temperature, agricultural fertilizer runoff, and anthropogenic pollution (Menon et al., 2023).

In semi-natural systems, knowledge of fish-pathogen-environment interactions is becoming more important in recent years especially in view of climate change, diminishing water quality, and growing human reliance on inland fisheries. Because reservoir fisheries lack the extensive management seen in aquaculture ponds, they are more susceptible to unrestrained disease spread (Bouwmeester et al., 2021). Maintaining fish health is critical not only for consumer nutrition and food safety but also for the economic viability of small-scale fisheries; hence, it is essential to evaluate the microbiological hazards and physiological effects of infection in wild environments, beyond controlled laboratory or hatchery conditions.

The present work attempts to close this information gap by separating bacterial pathogens from naturally occurring *Cypri*nus carpio in Limboti Dam, defining their enzymatic pathogenicity, and evaluating the effects of infection on the physiological and nutritional state of the host. This work aims to clearly relate pathogen burden to host degradation by including microbiological diagnostics, water quality monitoring, haematological evaluation, histological research, and biochemical analysis of muscle tissues. The results should offer fisheries departments practical information, guide the development of effective disease surveillance systems and promote sustainable reservoir fisheries management in Maharashtra.

2. Review of Literature

2.1 Importance of *Cypri*nus carpio in Inland Aquaculture

Cyprinus carpio is native to Europe and Asia and has been introduced to every part of the world except poles.They are the third-most frequently introduced fish species worldwide(Courtenay et al.,1989).It's adaptability,diverse feeding habits and ability to improve pond ecosystem health have helped it to be effectively included into polyculture systems in India. Unlike carnivorous species, it utilizes various food resources in a pond, including benthic organisms and organic matter, maximizing the productivity of the water body.

It is a preferred species in open-water fisheries and reservoirs due to its capacity to survive across a broad spectrum of environmental circumstances. But the rising accounts of mass mortality, slowed development, and illness outbreaks have underlined *Cyprinus carpio*'s vulnerability to bacterial infections—particularly in semi-managed or unmanaged water bodies such as Limboti Dam.

2.2 Overview of Bacterial Pathogens in Freshwater Fish

Freshwater microbial populations are highly diverse; some of them are harmful to fish. Among the most often isolated bacteria from sick freshwater fish are opportunistic species such *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Edwardsiella tarda*, and *Flavobacterium columnare* (Fryer & Rohovec, 2021 and Zaheen et al., 2022). Usually in the water as part of the regular flora, these bacteria can become harmful under environmental stress or immunosuppression.

Commonly linked in carp species to fin rot, hemorrhagic septicemia, and ulcerative cutaneous infections are *Pseudomonas fluorescens*. Similarly, *Edwardsiella tarda* is known to produce edwardsiellosis, a systemic disease defined by internal organ destruction, ascites, and granulomatous lesions in the liver and spleen (Pandey et al., 2021). Globally, both pond-based and reservoir-based aquaculture has suffered major financial losses attributable to these diseases (Kelkar & Aurther, 2022).

2.3 Virulence Mechanisms of Pathogenic Bacteria

The release of extracellular enzymes that support invasion, nutrition acquisition, and immune evasion defines bacterial pathogenicity. These agents comprise:

- Proteases - Proteases break down structural proteins such as elastin and collagen, therefore destroying connective tissues and generating localized necrosis (Mainardi, 2001).
- Lipases - Lipases break down host cell lipid membranes, therefore upsetting cellular integrity and setting up inflammation (Justine et al., 2017).
- Lecithinases (Phospholipases) - Particularly target phospholipids in cell membranes to cause cytolysis and widespread tissue damage (Baxter et al., 2017).

By lowering host immune responses, these enzymes not only encourage tissue death but also provide ideal circumstances for bacterial multiplication. Research on *Aeromonas* species has shown that the degree of sickness in cyprinid fish directly corresponds with the presence of several virulence genes, including those coding for aerolysin, hemolysin, and elastase (El-Bahar et al., 2019).

2.4 Physiological Responses to Bacterial Infections

Usually displaying significant alterations in hematological parameters, infected fish are sensitive markers of systemic illness and physiological stress (Chen & Luo, 2023). Commonly reported hematological changes include:

- Hemoglobin Reduction: Hemoglobin loss points to anemia and impaired oxygen flow.
- Reduction in RBC count and hematocrit: Indices hemolysis or inhibition of erythropoiesis.
- Altered leukocyte profiles: Raised WBC count may point to acute infection while leukopenia may imply persistent immunological suppression (Carli et al., 2015).

Lethargy, loss of appetite, aberrant swimming behavior, and more surface breathing—all indicators of compromised metabolic activity and respiratory discomfort—are further physiological expressions.

2.5 Histopathological Damage in Infected Fish

Key organs at the tissue level can exhibit pathogen-induced damage including:

- Gills: Hyperplasia, lamellar fusion, and epithelial lifting all compromise gas exchange and osmoregulation (Rasmi & Faisal, 2024).
- Liver: Affects detoxification and metabolism are fatty degeneration, necrosis, and vacuosity.
- Kidney: Tubular necrosis and glomerular degeneration compromise excretion and ion balance.

Such injury seriously reduces the fish's capacity for survival under natural reservoir circumstances and for maintaining homeostasis.

2.6 Nutritional Implications of Bacterial Disease

Fish's metabolic condition has direct bearing on their nutritional value. Extended bacterial infections result in:

- Protein catabolism: Particularly in cases of an anorexia lowering nutritional intake; the host catabolizes body proteins to satisfy energy needs (Olson et al., 2020).
- Lipid Depletion: Enhanced oxidative stress and lipid peroxidation cause infected fish to often show reduced lipid content (Ogueji et al., 2020).
- Loss of Micronutrient: Tissue deterioration and inflammatory reactions cause a depletion in water-soluble vitamins including B-complex and vitamin C as well as in fat-soluble vitamins including A and E (Rafeeq et al., 2020)

Bacterial stress in Cyprinus carpio causes noticeably decreased protein and fat content in muscle tissues, thereby influencing consumer nutrition as well as market quality.

2.7 Environmental Factors Affecting Pathogen Proliferation

For freshwater fish, disease dynamics are highly influenced by water quality (Svobodova et al., 2017). Temperature, pH, ammonia content, and dissolved oxygen especially influence:

- Bacterial Load: Warmer temperatures speed up bacterial development and virulence gene expression.
- Host Immunity: Low oxygen and high ammonia reduce immunological reactions and raise stress hormone levels, therefore influencing host immunity.
- Disease Transmission: Eutrophication and turbidity raise pathogen persistence and improve interaction between hosts and pathogens, therefore promoting disease transmission.

Due to inconsistent water level regulation and runoff from surrounding agricultural fields, reservoirs like Limboti Dam are very sensitive. These elements raise the microbial load and produce hotspots for infection in pre- and post-monsoon seasons.

2.8 Gaps in Regional Research and Justification for the Study

Although worldwide studies on fish bacterial infections have been conducted in great detail, regionally specific studies from inland reservoirs in Maharashtra are rare. With limited research addressing physiological or nutritional impacts, the few studies that are accessible—those by Pandey et al., (2017) concentrated mostly on pathogen identification and are limited to *Catla catla*.

Comprehensive studies examining pathogen load, virulence activity, hematological changes, histological damage, and dietary modification concurrently in Cyprinus carpio from an open reservoir such as Limboti Dam are lacking. This knowledge vacuum limits the evolution of locally relevant mitigating techniques and predictive disease models.

2.9 Emerging Focus on Integrated Fish Health Management

The integration of microbiological diagnoses with host physiology and environmental monitoring is underlined in present trends in aquatic health. For areas like Marathwada, where small-scale fisheries rely mostly on natural water bodies and lack disease forecasting tools, such frameworks are especially pertinent.

With a multidisciplinary approach, the current work intends to provide significant data for reservoir fisheries health management in Maharashtra and offer a model for future research in other inland systems in India.

3. Objective of the study:

A. To investigate the physiochemical properties of Limboti Dam,Dist.Nanded, Maharashtra.

B. To isolate and identify the bacterial pathogens associated with Cyprinus Carpio in Limboti Dam using morphological, biochemical, and molecular methods.
C. To investigate extracellular enzyme production viz. protease, lipase, lecithinase by these pathogens as an indicator of virulence.
D. To evaluate hematological parameters in infected versus healthy Cyprinus Carpio.
E. To examine changes in terms of protein, fat, carbohydrate, moisture, and vitamin content in nutritional context.
F. To examine how water quality factors influence pathogen transmission and infection severity.
G. To link physiological and nutritional deterioration with pathogen load and enzyme activity.

4. Hypotheses

Pathogenic bacteria significantly alter the haematological and nutritional profile of Cyprinus Carpio, with environmental stressors modulating the extent of these effects.

5. Materials and Methods

Using microbiological, biochemical, physiological, and environmental studies, this multidisciplinary approach will evaluate the health state of *Cyprinus carpio* (Common carp) in Limboti Dam. Standard biosafety and ethical handling practices for aquatic life will guide all treatments.

5.1 Site of Investigation and Sampling Strategy

Built for irrigation and fishery, Limboti Dam is a man-made reservoir in Nanded district of Maharashtra. To capture microbiological and ecological variability, the reservoir will be investigated periodically during one annual cycle—pre-monsoon, monsoon, and post-monsoon.

Cast and gill nets will be used to collect Cyprinus carpio specimens—both healthy and Infected. Selection of sick individuals will be guided by physical symptoms such skin lesions, fin rot, hemorrhagic patches, or slow behavior. Additionally, sampled for baseline comparisons are control fish free of outward indicators of illness.

5.2 Isolation and Enumeration of Pathogenic Bacteria

Aseptically swabbing tissues from contaminated bodily parts (liver, kidney, skin ulcers), they will be suspended in 0.85% saline for serial dilution.

- **Total Viable Count:** Under incubation at 28°C for 24–48 hours, spread plate technique on Nutrient Agar and MacConkey Agar.
- **Selective Isolation:** Gram-negative bacterium differentiation using XLD or EMB Agar
- **Purification:** Getting pure strains by subculturing morphologically different colonies

5.3 Identification and Characterization of Pathogens

- **Morphological and Biochemical Tests:** Preliminary characterization based on Bergey's Manual will utilize morphological and biochemical tests including gram staining, oxidase, catalase, urease, citrate usage, triple sugar iron, and MR-VP assays.
- **Molecular Identification:** 16S rRNA PCR will be used on genomic DNA from certain isolates. Sequenced and examined using BLAST, amplified products will be confirmed in identity.

5.4 Virulence Profile of Enzymes

Every bacterial isolate will be examined for exoenzyme synthesis to ascertain pathogenicity potential:

- Protease Activity: Agar skim milk; clear halos indicate casein hydrolysis.
- Lipase Activity: Tributyrin agar to measure lipid hydrolysis.
- Lecithinase Activity: Phospholipase detection egg yolk agar

Measurement and scoring of zones of clearing or opacity will help to evaluate intensity.

5.5 Analysis of Water Quality Parameters

Simultaneous collecting of water samples from every sampling location will be done for:

- pH and temperature (digital sensors)
- Dissolved Oxygen (DO) (Winkler titration)
- Ammonia (Calorimetric method using Nessler's reagent)
- Salinity and Conductivity (handheld meters)

Environmental factors will be linked to seasonal pathogen profiles and disease incidence.

5.6 Haematological Evaluations

Fish will be anaesthetized, and blood will be drawn from the caudal vein with syringes coated in EDTA-coating. The following parameters will be measured:

- Hemoglobin concentration (cyanmethemoglobin method)
- Red and white blood cell counts (manual hemocytometer)
- Packed cell volume (microhematocrit method)
- MCH, MCHC, MCV values will be calculated accordingly.

5.7 Histopathological Observations

Dissected and fixed in 10% buffered formalin, organs—gills, liver, kidneys—will be embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E). Photographs and documentation will be taken of pathological alterations like vacuosity, bleeding, or cellular necrosis.

5.8 Analysis of Nutritional Composition

- **Moisture and Ash Content:** Oven-drying and muffle furnace methods.
- **Crude Protein:** Estimated using Lowry or Kjeldahl method.
- **Total Lipids:** Extracted via Soxhlet with petroleum ether.
- **Carbohydrates:** Anthrone method for total sugar estimation.
- **Vitamin estimation** (subject to feasibility)**:** will be conducted using HPLC techniques.

5.9 Statistical Analysis

Statistical programs like SPSS or R: will be used as per the feasibility to examine data:

- Descriptive statistics for every criterion
- T-tests and ANOVA for a comparison between groups (healthy and infected)
- Pearson correlation for bacterial load against changes in physiology and diet
- Significance level set at $p < 0.05$

6. Current Status of Research

6.1 International Status

Because of its economic importance and sensitivity to bacterial infections in intensive freshwater aquaculture systems, *Cyprinus carpio* has been the focus of much study worldwide. Large-scale studies on disease epidemiology, pathogen diversity, and vaccination development have looked upon *Cyprinus carpio*, one of the most often grown freshwater species, in China . Key virulence genes including aerA, hly, and act linked with host tissue degradation and systemic infection were found by research by Ahangeazadeh et al. (2022) and Abdella et al. (2024) documenting epidemics produced by *Aeromonas hydrophila* and *Edwardsiella tarda*.

Using molecular techniques, the function of extracellular enzymes such as proteases and lipases in virulence has also been well investigated; real-time PCR and proteomics-based approaches have helped to identify a relationship between environmental stress and virulence factor expression (Selvaraj et al., 2020).

Pathogenesis in Cyprinus carpio has been investigated by fish cell culture models (Zhi et al., 2022). Moreover, comprehensive health management—including the use of probiotics, immunization, and environmental monitoring—has become a regular feature of intense *Cypri*nus carpio farming However, such advancements are yet to be widely adopted by emerging inland fisheries (Manam et al., 2024. fisheries industries have not embraced such advances yet.

6.2 National Status

Research on freshwater fish diseases has mostly concentrated in India on Indian main carps such *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*, with rather little attention paid to alien (Exotic) species like *Cyprinus carpio* (Sandilyan, 2022 and Singh, 2024). Although the species has been effectively included into polyculture systems, systematic research on its health under reservoir settings are few.

Most attempts at disease surveillance focus on hatcheries and commercial ponds, where occasional reports of *Aeromonas*, *Pseudomonas*, and *Flavobacterium* have been made (Chitambo, 2021 and Verma, 2023). Though these efforts lack species-specific research on nutritional and physiological impacts in natural water bodies, ICAR-CIFA and regional veterinary universities have helped to understand the bacteriology of Indian aquaculture systems (Sethi et al., 2023 and Dhere et al., 2025).

Particularly in the Marathwada area of Maharashtra, relatively few records of fish sickness from open reservoirs exist. One of the few studies showing the existence of *Pseudomonas fluorescens*

in *Catla catla* is (Verma, 2023); it does not evaluate the physiological or nutritional consequences of such infections or include *Cyprinus carpio* either (Kumar et al., 2022 and Wildhaber et al., 2023). Furthermore, disease outbreaks in reservoirs such as Limboti Dam typically involve localized responses without microbiological validation or post-outbreak analysis.

For fish health in inland Indian reservoirs, there is no known surveillance system. Native Cyprinus Carpio populations are especially vulnerable to unmanaged disease progression due to a lack of organized research and environmental problems including eutrophication and human runoff.

7. Complete Work Plan and Timeline

Year	Key Activities
Year 1 2025-26	• Completion of PhD coursework • Literature survey on grass carp pathology and bacterial virulence
	• Field visits to Limboti Dam for sampling site mapping • Seasonal recording of water parameters (pH, Temperature, DO, Ammonia) • Collection of grass carp specimens (healthy and infected)
Year 2 2026-27	• Initial isolation and culture of bacterial strains • Morphological and biochemical identification of bacterial isolates • Molecular identification via 16S rRNA PCR • Article Publication

Year	Key Activities
Year 3 2027-28	• Enzymatic profiling (protease, lipase, lecithinase) • Laboratory experiments on pH, salinity, and temperature impact on isolates • Haematological testing of infected and control fish (Hb, RBC, WBC, PCV) • Histopathological analysis (gills, liver, kidney) • Nutritional composition assays: protein, lipids, carbohydrates, moisture, ash, and vitamins • Article Publication
Year 4 2028-29	• Integration and analysis of all datasets • Chapter-wise writing of the PhD thesis • Preparation of graphs, tables, and histological images • Final formatting, submission, and defense presentation

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Tamke Sneha Sanjay **Dr. R. D. Barde**